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Comparative Anatomy of Certain Hybrid Shrubs and Their Parents.

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THE study of transmitted characters in hybrid offspring has become the chief method of determining the laws of heredity. Usually, since the rediscovery of Mendel's law, this study has been one of quantitative and qualitative data in plant- and animal-breeding where clearly recognized characters were observed for several generations. Many cytological studies have also been made of hybrids, especially during recent years. Anatomical studies of plant hybrids have not been so common.

Henslow ('31) compared a hybrid *Digitalis* with its parents in a very minute way, considering the knowledge of plant anatomy of the time. He found in the size and shape of the hairs and other structures the hybrid was intermediate between those of its parents.

Wettstein ('88) compared the leaves of four coniferous hybrids and found them exactly intermediate between their parents in number of stomata, depth of epidermal cells, and number and arrangement of the sclerenchyma cells of the bundles.

Noble ('88) compared *Clematis jackmanni alba* with its parents and found it had flowers resembling both parents. He explained that the *C. patens,* its spring-flowering parent, and *C. jackmanni,* its autumn-flowering parent, seem to set up a kind of rivalry to see which is stronger. Old wood of *C. jackmanni alba* in May, June and July bears double and semidouble solitary flowers of a bluish French-grey color, as the *C. patens* parent does; and then young shoots of the hybrid bear single white flowers in pairs on a long raceme, in August and September, as the *C. jackmanni parent* does. At one age of the wood it resembles one parent, and at a later age it resembles the other parent.

Hildebrand ('89) found in a cross between *Oxalis latifolia* and *O. tetraphylla* that the characteristically distinct hairs of each species

might both arise from a single epidermal cell of the offspring. Both parents seem to be contributing to a single factor.

Branza ('90) compared the tissue masses of certain seed hybrids and of *Cytisus adami,* a graft hybrid, with those of their parents. Since his observations differ in many details from those of MacFarlane ('90, '91), they are discredited.

The most extensive and intensive study of plant hybrids in relation to their parents was made by MacFarlane ('90, '91). He compared the minute structure of the following hybrids with that of their parents: *Philageria veitchei, Dianthus grievei, Geum intermedium, Ribes culverwelli, Saxifraga andrewsii, Erica watsoni, Bryanthus erectus, Masdevallia chelsoni, Cypripedium leeanum,* and *Cytisus adami* (a graft hybrid).

His conclusions are summed up in these words: "We may recall the facts advanced as to color, flowering period, chemical combinations, growth, and vigor which, though scanty and fragmentary in their nature, all point to the conclusion that hybrids are intermediate between their parents in general life phenomena." On the divergence of some hybrids or parts of hybrids toward one parent, he explains it on a purely physical basis of under or overnutrition, or through advantageous or disadvantageous position. With the exception of this conclusion, which is now explained in a very different way, his study constitutes a great contribution to the study of heredity and is so regarded by the editor of the Gardener's Chronicle ('93).

Henslow ('93), in a study of *Rhododendron* hybrids, found that one parent often impresses some peculiarity which he calls sexual hybridity, but that as a general rule characters are more or less intermediate between those of their parents.

Farmer ('97), in studying a hybrid fern, decided that inheritance is the result of an imaginary struggle between purely hypothetical combatants, and thus accounts for the resemblance of this or that cell to corresponding cells in one or the other parent.

The rediscovery of Mendel's law in 1900 explained in a new way the tendency of hybrids to resemble one or the other parent as due to dominance and recessiveness of characters. The studies in this field since that time have been largely of the plant or animal-breeding type when characters were traced through several generations.

MacDougal ('07), in a study of hybridization of wild plants states that anatomical study of a hybrid and the parents to which it has been referred would be a good method of determining whether

or not it is a true hybrid. He cautions that this may not be decisive because of a tendency toward one parent.

De Vries ('10) calls those qualities which in crossing conform to Mendel's law bisexual or varietal characters, and those which give intermediates unisexual or specific characters. He states, "The points of difference between parents can be all unisexual or all bisexual, or some of them may be unisexual and others bisexual. In the first case the parents are to be considered as elementary species; in the second as varieties; in the third, however, the principle affords no decision." (p. 587.) He further states that in experiments in hybridization we must "confine our attention to certain points of difference and leave all the rest out of consideration as of subordinate importance. If the character is bisexual and behaves in a Mendelian fashion we may immediately infer that the two parents are to be considered as varieties. If it is unisexual they are elementary species, of which the one must have been derived from the other . . . by modulation."

East ('12) chose flowers in *Nicotiana*. After proving that characters were uninfluenced by environment he found that these characters were intermediate in the F_1 generation. Among the flowers in the F_2 generation he found also flowers identical with those of each parent.

Molloch ('21) found in a cross between *Hordeum vulgare* and *H. murinum*, two species which differ in a large number of morphological characters, that the hybrid died, probably due to the failure to harmonize between the reactive systems of the two species. He states, "There are all degrees of incompatibility of reaction systems in species crosses."

Tedin ('23) found that two forms of *Camelina*, differing from each other in almost every character, had probably two characters that influenced leaf shape. The interpretation was that AABB = pinnatified leaves; aabb = entire leaves; AAbb = pinnatified with shorter and broader lobes than aaBB = deeply denate single leaves.

Anyone who has undertaken a comparison of hybrids must agree with MacFarlane in his statement that, "When a hybrid is the product of parents that are widely divergent in histological details the comparison will be easy, but when we attempt to compare a hybrid with two parents which are regarded as species, but whose chief specific differences are those of coloring and size, it is almost or quite impossible to detect any blending or parent characters even though these may occur."

The histological study of hybrids has generally been considered a good means of verification of doubtful hybrids. It has not always been so fruitful for determining the laws of heredity.

Davenport ('07) states that, "The common mistake has been to note a few remarkable individuals and exceptional instances, and from these attempt to deduce the 'laws of descent.' To determine the facts of heredity with any degree of reliability we must study the race as a whole and not simply the separate individuals that compose it. The laws of descent are to be discovered by a critical study, not of individuals but of entire populations sufficiently large to be safely representative. Unfortunately, the application of the statistical method to the study of this subject is comparatively new, and as it is extremely laborious, the accumulation of a large mass of material will of necessity be a somewhat slow process." (p. 478.)

The study of the detailed anatomy of whole races of hybrids would be quite worth while, and with the advance of our knowledge of minute structure of plants and the perfecting of our methods of studying them it will some day no doubt be attempted. There are certain features which are modified greatly by differences in environment and the age of the plant. Great care must be exercised in comparing these features.

Since shrubs offer good subjects for anatomical study and many hybrids have already been produced among them, I have chosen from them for this comparative study.

The following shrubs with their parents were secured for comparison:

Berberis neubertii = *B. vulgaris* × *Mahonia aquifolia.*
Syringa chinense = *S. vulgaris* × *S. persica.*
Forsythia intermedia = *F. suspensa* × *F. viridissima.*
Lonicera bella = *L. tatarica* × *L. morrowi.*
Lonicera notha = *L. ruprechtiana* × *L. tatarica.*
Lonicera muendiensis = *L. bella* × *L. ruprechtiana.*
Lonicera muscaviensis = *L. morrowi* × *L. ruprechtiana.*

From the cross and longitudinal sections of the stems of these hybrids and their parents it was apparent that *Berberis neubertii* and its parents offered the most points of difference. It was therefore selected for the major part of this study.

The origin of *B. neubertii,* which is usually considered an intergeneric hybrid, is described in L'Illustration Horticole as follows: "Il a ete trouve, en 1850, dans un jeune plant issu de graines recueillies sur un *Berberis atropurpurea* et sur un *Berberis* (*Mahonia*) *aquifolia,* croissant tous deux l'un pres de l'autre, par M. Aug. Nap.

Baumann, horticulteur a Bolwiller (France), et est evidement le resultat d'un croisement artificiel entre ces deux especies, si diverese par le port; croisement opere par quelque insecte butineur."

The *B. neubertii* plant from which this study was made was secured from Farquhar, who secured it from Arnold Arboretum. In general characteristics of leaf and stem it agrees with the description and illustration in L'Illustration Horticole, the illustration in Gardener's Chronicle, and the description in Bailey's Encyclopedia of Horticulture, but differs in two very important details. My specimen is spined, but the descriptions and illustrations give it as spineless. The leaves are grouped as those in *B. vulgaris* in the axils of the spines, while the descriptions give them as somewhat sheathing the stem as described in *Mahonia aquifolia*. The second difference may be the result of the first, which is probably a reversion to one of its parents, *B. vulgaris*. Farquhar stated in a letter that he did not have a spineless plant but that as far as he knew his plants were true hybrids. The only explanation for the decided change in the hybrid since it was first produced in 1850 would be that it has reverted back in this respect to one of its parents, in this case *B. vulgaris*.

Farmer, in his study of a hybrid fern, found that it had a tendency to revert to one or other of its parents in certain particular characteristics, and plants obtained from cuttings of such branches grew and remained true. The reverted branch did not in every particular resemble one parent, but this parent became more strongly pronounced. He considered this a striking illustration of the unstable character of a hybrid.

The determination of the true hybridity of my specimen was therefore my first problem. As will be observed later, it agrees with what we may expect in a true hybrid.

DISCUSSION.

Mahonia (sometimes called *Berberis*) *aquifolia* is an evergreen, broad-leaved spineless shrub with odd-pinnate leaves. It has minute subulate stipules. The base of the leaf is sheathing, from one-half to completely surrounding the stem. The glossy leaves of firm texture give it a stiff xerophytic appearance. Its habit of growth is by sudden limited, tender, terminal shoots which are later strengthened. *Berberis vulgaris* is a deciduous, spined, simple-leaved shrub. The spines are morphologically leaves and the foliage leaves are borne on short branches in their axils. The growth is more gradual and indefinite than that of *Mahonia* and the branches are more

pendulous and graceful. The texture is less firm and the appearance truly mesophytic. It has very minute subulate stipules, and below them the petiole is flattened and partly sheathed and indistinctly jointed.

The hybrid, *B. neubertii,* is classed as a semi-evergreen in the habitat of Boston. My specimen lost most of its leaves with the first snow, but a few leaves still clung to the branches. The spines are similar to those of *B. vulgaris* and the foliage leaves are borne on short stems in their axils. The growth is indefinite, resembling that of *B. vulgaris,* but it has a tendency toward the stiff appearance of *Mahonia.* It has very minute stipules and jointed petiole, but the petiole is much larger than that of *B. vulgaris.* The general appearance may thus be said to be intermediate between that of its parents.

Since *Mahonia aquifolia* has a compound leaf and *Berberis vulgaris* a simple leaf, the hybrid would necessarily follow one or the other of these characters. The hybrid has simple leaves showing the dominance in kind of leaf to be that of its parent, *B. vulgaris.* In many details it is clearly influenced by the leaf of *Mahonia.*

In shape the *Mahonia* leaflet (Pl. XXXVII, Fig. 3) is oblong-ovate, sessile, or nearly so, and the sides of the base are unequal. The leaf of *B. vulgaris* (Pl. XXXVII, Fig. 1) is oblong-spatulate with a petiole 8-10 mm. long and indistinctly jointed near the base. *B. neubertii* has a leaf (Pl. XXXVII, Fig. 2) of a similar shape to that of *B. vulgaris,* but the petiole is intermediate in both breadth and length between the petiolule of *M. aquifolia* and the petiole of *B. vulgaris.* The stipules are very similar to those of *B. vulgaris.* The sheathing base of the petiole below the stipules and joint is much broader in the hybrid than in that of *B. vulgaris,* showing a tendency toward the base of the leaf of *M. aquifolia.*

The margin of the leaflet of *M. aquifolia* is spinulose-dentate with lobes 5-10 mm. apart. There is a prominent veinlet for each lobe. *B. vulgaris* has a setulose-dentate leaf with the lobes 1-1.2 mm. apart, and a small veinlet for each lobe. *B. neubertii* has a spinulose-dentate leaf and thus clearly follows *M. aquifolia,* but exceeds it in the size and distance apart of the lobes. On the same branch, the leaves of *B. neubertii* are quite variable in the size and distance apart of the lobes. They have a prominent veinlet for each lobe. The spines of the margin are very similar in length and texture to those of *M. aquifolia,* being much more rigid than those of *B. vulgaris.* The spines in all cases include continuations of the group of schlerenchyma cells which form the margin of the leaves.

Sclerenchyma cells are fewer in number in *B. vulgaris* and therefore the spines of the leaf are less rigid.

In Pl. XXXVII, Figs. 1, 2, 3, the general type of venation of the leaves may be observed. *M. aquifolia* (Pl. XXXVII, Fig. 3) has 5 to 6 primary lateral veins and between each of these 3 to 6 veinlets from the midrib. The type of venation agrees with what we might expect from the shape of the leaf. *B. vulgaris* (Pl. XXXVII, Fig. 1) has 3 to 5 primary lateral veins and between each of these as many as 16 small veinlets from the midrib. In venation *B. neubertii* (Pl. XXXVII, Fig. 2) agrees very generally with *B. vulgaris,* but has fewer veinlets from the midrib, following after *M. aquifolia* in this respect.

The color of the leaf of *M. aquifolia* is a yellowish green, and lustrous when young, but dark green and lustrous when mature. The leaf of *B. vulgaris* is a pale or grayish green beneath but a dark green above. It is dull in appearance. The leaf of the hybrid agrees with that of its *B. vulgaris* parent in color.

Upper epidermis cells, as shown in Pl. XXXVII, Figs. 4, 5, 6, show the lateral walls in the leaf of *B. vulgaris* (Pl. XXXVII, Fig. 4) to be symmetrical. Corresponding cell walls in the leaf of *M. aquifolia* are very sinuous or undulated (Pl. XXXVII, Fig. 6). The hybrid (Pl. XXXVII, Fig. 5) has epidermal cells that are very similar in shape to those of *B. vulgaris,* but the size of the cells is greater than in either parent.

The stomata in both parents and the hybrid are found only in the lower epidermis of the leaf. They agree in shape and size but differ in number. In *M. aquifolia* I found by count the average number to be 260 per sq. mm. In *B. vulgaris* the average number was only 176. The mean number between these two is 218. The average number in the hybrid was found to be 216 per sq. mm. This agrees with MacFarlane's observation of *Philageria Veitchii* and other hybrids, in which the hybrid has an intermediate number of stomata between that of its parents, respectively.

The cross section of the leaf cut through the two guard cells offers a basis of comparison. The guard cells of *B. vulgaris* (Pl. XXXVII, Fig. 10-H) are closely followed by those of *B. neubertii* (Pl. XXXVII, Fig. 11-H). The guard cells of *M. aquifolia* (Pl. XXXVII, Fig. 12-H) are somewhat external to other epidermal cells.

The lower epidermal cells of the leaf and the epidermal cells of the petiole in *M. aquifolia* show a network of thickenings (Pl. XXXVII, Figs. 9-F, 12-H, and Pl. XXXVIII, Fig. 13-F). This net-

work was very conspicuous in mature leaves, but was not seen in young leaves. *B. vulgaris* has no such thickenings in the epidermal cells (Pl. XXXVII, Figs. 7 and 10, and Pl. XXXVIII, Fig. 15-F). The hybrid has a very indistinct network of thickenings in mature leaves only (Pl. XXXVII, Fig. 8-F). This character, which behaves as an intermediate, was one of the most convincing proofs of the true hybridity of my specimen.

In the cross section of the leaves (Pl. XXXVIII) there are two distinct rows of palisade cells in the leaf of *M. aquifolia.* The third row of cells is apparently intermediate between palisade and spongy parenchyma cells (Pl. XXXVIII, Fig. 13). *B. vulgaris* (Pl. XXXVIII, Fig. 15) has but one row of palisade cells. The environmental conditions of the two plants was as nearly the same as could be secured. The hybrid very clearly follows the *M. aquifolia* parent in the number of rows of palisade cells (Pl. XXXVIII, Fig. 14), but follows the *B. vulgaris* parent in the depth of palisade cells. The greater thickness of the leaf of the hybrid than that of either parent is thus in part accounted for.

The number of rows of spongy parenchyma cells of the three leaves shows the hybrid to have more rows than either parent. The *M. aquifolia* parent has from 5 to 6 rows of spongy parenchyma. *B. vulgaris* has from 4 to 5 rows. The hybrid has from 7 to 9 rows. The size of the cells in the hybrid is also greater than in either parent. Since all of these plants were growing under similar environmental conditions, and the above-described condition appears as a constant characteristic, it may be considered as inherited.

Cross sections of the leaves cut through the midrib in various positions in the leaf offers a basis of comparison. In Pl. XXXVII, Figs. 1, 2, 3, the letter A indicates a position in the midrib in which the bundle is single, with schlerenchyma extending to both the upper and lower epidermises. At B the bundle has divided into three, at C it has divided into 5, and at D it has divided into 9 bundles (Pl. XXXVII, Fig. 3 only). By a comparison of the positions of A, B and C in the three figures, it will be observed that the hybrid clearly follows the *B. vulgaris* parent in this character.

From cross sections of the petiole of *B. vulgaris* and the petiolule of *M. aquifolia,* the number of rows of thin-walled parenchyma cells surrounding the vascular area formed a basis of comparison. In the *B. vulgaris* parent there are from 3 to 5 rows of thin-walled parenchyma cells surrounding the vascular area of the petiole. The petiolule of *M. aquifolia* has from 4 to 6 rows of cells in this area

The hybrid has from 5 to 7 rows. The hybrid thus exceeds the number of rows of both parents, which partly accounts for the larger petiole than in either parent. The petiole of *M. aquifolia* differs from the others in that the schlerenchyma cells form a complete ring around the vascular area. Near the base of the petiole the bundles again form a semicircle conforming with the sheathing base of the leaf. In *B. vulgaris* the vascular area never forms a complete ring, but somewhat more than a semicircle. The hybrid follows the *B. vulgaris* parent in this character.

A comparison of the prominence of the midrib below the blade of the leaf shows that in both parents the midrib is very prominent. The hybrid has a relatively thicker blade than either parent, and thus a relatively less conspicuous midrib below the blade of the leaf. The small veins of the leaf in the hybrid scarcely exceed the thickness of the blade, while in both parents they are prominent on the under side of the leaf.

Papillose epidermal hairs occur very generally on the lower epidermis of the leaf and always on the young stem of the *Mahonia aquifolia* parent. They average 8μ in height and 12.5μ in diameter. No epidermal hairs were found on the leaves or young stems of *B. vulgaris* nor on the hybrid. This character behaves in this instance as a recessive, but no conclusion may be drawn from this one hybrid.

Epidermal hairs were observed in *Lonicera morrowi* on leaves and young stems. On the stems they are very irregular as to length varying from .08 mm. to .48 mm. The number counted was 360 per sq. mm. of epidermal surface. In *L. tatarica* usually no epidermal hairs are found, although they were observed on a few young leaves. The hybrid from these parents, *L. bella,* has epidermal hairs varying in length from .04 mm. to .32 mm., and averaging 120 to the sq. mm. It appears in this that the hybrid has the epidermal hairs of *L. morrowi* reduced by half in number and size.

Further study of the epidermal hairs in the honeysuckle showed for *Lonicera muscaviensis,* a hybrid of *L. ruprechtiana* and *L. morrowi,* that epidermal hairs were intermediate in size, shape, and number between those of the parents. *L. ruprechtiana* has epidermal hairs varying in length from .08 mm. to .32 mm. and averaging 76 per sq. mm. of surface. The intermediate number between the parents would be 218. The hybrid averaged on several counts 160. The intermediate length between the parents would be from .08 mm. to .8 mm. The length of the epidermal hairs of the hybrid was found to vary from .08 mm. to .32 mm.

Lonicera notha, a hybrid between the parents *L. tatarica* and *L. ruprechtiana,* shows also an intermediate number and length. *Lonicera muendiensis,* a hybrid between *L. bella* and *L. ruprechtiana,* also has hairs showing an intermediate size and number. The shape of the epidermal hairs also shows the influence of both parents. These examples all point to an intermediate inheritance of epidermal hairs in *Lonicera.*

Hildebrand found that epidermal hairs in the hybrid of *Oxalis latifolia* x *O. tetraphylla* were inherited from both parents, both kinds being on a single cell of the hybrid.

MacFarlane found that in the hybrids *Ribes culverwelli, Saxifraga andrewsi,* and *Carduus carolorum,* distinct types of epidermal hairs were inherited from both parents. These are considered examples of bisexual heredity by MacFarlane.

It seems that no general rule obtains in the inheritance of epidermal hairs.

The number of stomata on the young stems of *Berberis neubertii* is much less than in either parent. In both parents the number averages about 23 per sq. mm., back where the stem has ceased to elongate. The hybrid averaged but 9 per sq. mm.

The size of epidermal cells on young stems showed the hybrid to be intermediate between that of its parents. *M. aquifolia* has cells averaging .08 mm. in length and .02 mm. in width after the stem has ceased to elongate. *B. vulgaris* has cells averaging .04 mm. x .02 mm. The hybrid has cells averaging .06 mm. x .02 mm., the intermediate between the parents.

The number of perforations in the lateral cell walls of the epidermal cells shows the hybrid to have a number below that of either parent. Pl. XL, Fig. 24-A, shows these perforations in the cell walls of *B. vulgaris.* No drawing was made of the similar cells of *M. aquifolia.* The drawing in Pl. XL, Fig. 26-A, shows the perforations in the lateral cell walls of the hybrid, much fewer in number than in either parent.

The cortex of the stem in *M. aquifolia* consists entirely of parenchyma cells. There is no distinct endodermis separating the cortex from the pericycle. Solereder has considered the primary bast-fiber ring as part of the pericycle. There is no typical collenchyma. The parenchyma cells of the cortex are large, averaging .03 mm. in width and .06 mm. in length. The cell walls are irregularly thickened with numerous perforations. The average number of rows of cortex cells was 7.2. The cortex of *B. vulgaris* also consists entirely of

parenchyma, but is always thin-walled. The average diameter of these cells was found to be .03 mm. and the length was found to be .13 mm. The walls are not perforated and not thickened as in the *M. aquifolia.* The cortex cells of young stems in *B. vulgaris* contain numerous disk-shaped chloroplasts. The number of rows of cells is irregular in conformity with the angled stem and the irregular bast-fiber ring. The number of cells radially may be as small as 2, but where the bast-fiber ring indents it may be 8 cells. The average number of rows was found to be 4.4 radially.

The cortex of the stem in the hybrid in some cases follows one parent and in others the other parent. The cell walls in *B. neubertii* have perforated and irregularly thickened cell walls, thus following the *M. aquifolia* parent. In size the cells are intermediate between that of the parents, being .1 mm. in length and .03 mm. in width as compared with an intermediate of .095 mm. in length and .03 mm. in width between the two parents. The general arrangement of the cells of the cortex in the hybrid is very similar to that found in the *B. vulgaris* parent, conforming to the angled stem and the irregular bast-fiber ring. The number of rows of cells radially in the hybrid is less than in either parent. (Pl. XXXIX, Figs. 17-B and 20-B for the hybrid, 16 and 19 for *M. aquifolia,* and 18 and 21 for B. *vulgaris.*)

The primary bast forms the outer part of the pericycle in both parents and the hybrid. In the *M. aquifolia* parent the bast-fiber ring is usually discontinuous in the stem, as shown in Pl. XL, Figs. 22-C and 25-C. The number of fibers may vary from a small number as shown in Pl. XXXIX, Fig. 19-C, to many as shown in Pl. XL, Fig. 22-C. The primary bast in *B. vulgaris* forms a continuous ring around the stem until it is broken by the growth of the stem. The number of cells radially may vary from 3 cells in the depressions to 12 cells at the angles of the stem. The bast-fiber ring is persistent for a relatively longer time than in *M. aquifolia* partly because of the continuous ring and partly because of undulations which allow for growth from within by straightening out as the stem increases in size. The hybrid very clearly follows the *B. vulgaris* parent in the arrangement of the cells of the primary bast of the stem (Pl. XXXIX, Fig. 20, Pl. XL, Figs. 23-C and 26-C). In number of rows of cells radially the hybrid exceeds that in either parent, showing 7.9 cells average for the hybrid and 6.3 cells in *B. vulgaris.*

The size of the primary bast fibers in the hybrid very closely approaches the intermediate between that of the parents. The length

for *M. aquifolia* averaged .8 mm, that for *B. vulgaris* 1.1 mm., the hybrid being 1.0 mm., which closely approaches the mean of .95 mm. For comparative length of bast fibers, see Pl. XLI, Figs. 31-E, 31-F, and 31-G, which represent average length of bast fibers in *M. aquifolia,* the hybrid, and *B. vulgaris* respectively. The diameter of individual bast fibers measured at the widest area shows also an intermediate size. The *M. aquifolia* parent averages 22μ in diameter, *B. vulgaris* averages 25μ in diameter. The diameter of the bast fibers in the hybrid averages 24μ, which closely approaches the mean between the parents, 23.5μ.

Macerations were made of primary bast by putting small sections into chromic acid. Other sections were placed in nitric acid and potassium chlorate and heated in a test tube, and thrown into water at the proper time to stop action. Individual bast fibers are shown in Pl. XLI, Figs. 31, 32, 33. Circular pits are found in the primary bast of *M. aquifolia* as shown in the surface view by rings and in the side walls as areas not darkened. *B. vulgaris* primary bast fibers have circular pits and elongated pits running diagonally across the fibers. The hybrid has both the circular pits, as shown in both parents, and also the diagonal pits as found in the *B. vulgaris* parent only.

The primary bast of *M. aquifolia* has irregular thickenings not dissolved by nitric acid as shown in black in Pl. XLI, Fig. 31, A and C. Other deposits within the cell are soluble in nitric acid. These deposits were not found in the *B. vulgaris* parent. The hybrid has deposits soluble in nitric acid in greater abundance than in the *M. aquifolia* parent. The irregular thickenings not soluble in nitric acid were not observed in the hybrid. Thus one unisexual character was inherited but the other was not.

Cork arises in the outer parenchyma of the pericycle just beneath the primary bast in both parents and the hybrid. In rapidly growing stems of *M. aquifolia* the cork arises in the seventh internode or thereabout. In *B. vulgaris* cork arises as early as in the second internode. In the hybrid cork was found in the second internode, thus showing this character to be inherited from the *B. vulgaris* parent. The growth of cork in the *M. aquifolia* parent is irregular because of the discontinuous bast-fiber ring. Cork arises both beneath and between the groups of bast, thus completely cutting them off. The epidermis and cortex of the stem hold several bast groups together, but between these groups they break apart in large longitudinal strips, a fact recognizable at the surface. In the *B. vulgaris* parent

the bast-fiber ring is continuous and the cork arises beneath it as a continuous ring, later cutting it off. The bast breaks at the narrower places but still persists as narrow longitudinal strips on the outer bark. The hybrid shows a very close resemblance to the *B. vulgaris* parent in the occurrence and growth of cork and its effect upon the stem.

The parenchyma of the pericycle was studied from cross sections of the stems. In *M. aquifolia* the cell walls of this area are unevenly thickened and perforated and the cells retain their shape, though flattened somewhat, for many years (Pl. XL, Figs. 22-E and 25-E). I did not find any sections in which these cells were crushed. The cell walls of this area in the stem of *B. vulgaris* are somewhat thickened, although not as much as in *M. aquifolia*. The hybrid very closely follows the *M. aquifolia* parent in the irregularly thickened and perforated cell walls.

The number of rows of cells radially in the parenchyma of the pericycle shows a very irregular number in the *B. vulgaris* parent due to the undulations in the bast-fiber ring. Later, as the stem grows and the undulations in the bast-fiber ring become straightened out, the corner areas are flattened and the narrower places in the parenchyma are elongated radially, with many intercellular spaces forming, thus making a somewhat uniform width of parenchyma cells of the pericycle. The *M. aquifolia* has a fairly uniform area of pericycle and maintains it. The hybrid clearly follows the *B. vulgaris* parent in the arrangement of cells in the parenchyma of the pericycle (Pl. XXXIX, Figs. 17-D and 20-D). In number of rows of cells radially, the hybrid has an average of 6, closely following *M. aquifolia*, which has from 5 to 6 rows. *B. vulgaris* has an average of 9 rows of these cells radially.

The primary phloem of all three consists of sieve tubes and also of septate prosenchyma cells tapering at both ends. These tapering ends overlap and the side walls are pitted or have thin areas. No distinct differences were observed in the primary phloem of the parents or the hybrid except the amount of primary phloem in proportion to primary xylem is greater in *M. aquifolia* than in *B. vulgaris* or the hybrid. The latter two are very similar. Also numerous intercellular spaces are formed in the older phloem by the breaking apart of the cell walls showing in the walls on edge as lenticular intercellular spaces. These intercellular spaces are less frequent in the *B. vulgaris* parent than in *M. aquifolia*. The hybrid is very similar to *B. vulgaris* in the number of intercellular spaces

appearing in the septate prosenchyma of the phloem, but the walls are thickened more nearly like those in *M. aquifolia.*

No secondary bast fibers were observed in *M. aquifolia.* The cell walls of the septate prosenchyma are perceptibly thickened (Pl. XXXVIII, Fig. 35) and serve a similar function of strengthening as is served by secondary bast. In *B. vulgaris,* secondary bast fibers resembling rod cells occur singly in the outer phloem, but in tangenital rows in the inner phloem (Pl. XL, Fig. 27-D). This may be considered a unisexual character as it occurs in only one parent. In the hybrid secondary bast fibers are found in greater abundance than in *B. vulgaris.* Pl. XL, Fig. 26-E shows these bast fibers just before the walls are thickened. They are completely thickened by the end of the second growing season. Pl. XL, Fig. 26-O, shows a cross section of these mature bast fibers. The hybrid is thus dominated in the occurrence of secondary bast by the *B. vulgaris* parent, but exceeds it in amount of bast.

No differences were observed in the primary xylem of all three subjects as studied from cross and longitudinal sections and macerated sections of stems. It is surrounded toward the pith by parenchyma with thickened walls, septate prosenchyma, and prosenchyma with pointed ends and thickened walls. There is more of this bundle sheath in *B. neubertii* than in either parent as shown in Pl. XL, Fig. 23-P, as compared with Pl. XL, Figs. 22 and 27. These cells are usually stored with food and the cell walls are thickened and pitted. In *B. vulgaris* the amount of thickening of cell walls is less than in *M. aquifolia.* The hybrid follows the *M. aquifolia* parent in the thickening but exceeds it in the amount of sclerenchymatous medullary sheath abutting on the primary xylem.

Cross, longitudinal, tangenital, and macerated sections were made of the xylem areas of the three subjects. The secondary xylem is made up in all three of tracheal tubes, tracheids, wood fibers, wood parenchyma, and septate prosenchyma. All of these cells have thickened walls and pits. The tracheids have bordered pits, and the tracheal tubes have both elongated pits as shown in Pl. XLI, Fig. 28-B, and circular pits as shown in Pl. XLI, Fig. 29-B. The circular pits have a narrow slit opening as shown in Pl. XLI, Figs. 29-B and 30-B. Many of the smaller tracheal tubes have spiral thickenings of the wall in addition to bordered pits, which occur in all three subjects, but especially in *M. aquifolia.* The comparison of the xylem elements is difficult because of the great similarities between the three subjects.

The tracheal tubes of the secondary xylem in *M. aquifolia* are relatively smaller and fewer than in *B. vulgaris*. The hybrid has tracheal tubes intermediate in size between those of the parents but more nearly approaching the number of *B. vulgaris*. Compare Pl. XL, Figs. 25-I, 26-I, and 27-I.

Wood fibers from macerated tissues of all three subjects show a general similarity in shape and pits. The *M. aquifolia* parent has wood fibers somewhat longer than those of the *B. vulgaris* parent. The wood fibers of the hybrid are intermediate in length between those of the parents. The average length of *M. aquifolia* wood fibers was found to be .38 mm., of *B. vulgaris* .34 mm., and of the hybrid .36 mm.

The tracheids are found in groups bordering either a group of wood fibers or the medullary rays. The average length of tracheids of the *M. aquifolia* parent was found to be .22 mm., in *B. vulgaris* average was .27 mm., and in the hybrid .247 mm. This very closely approaches the intermediate length between those of the parents, .254 mm.

The wood parenchyma is very little developed in the *M. aquifolia* parent, is more common in the *B. vulgaris* parent, and still more common in the hybrid. A definite comparison is difficult to make as to amount of wood parenchyma.

The wood prosenchyma with delicate cross walls across the lumen is common to all three subjects, but more common to *B. vulgaris* than to *M. aquifolia*. The hybrid follows the *B. vulgaris* parent in this character.

Broad primary medullary rays separate the individual vascular bundles from each other in all three subjects. The cell walls are perforated as shown in Pl. XLI, Figs. 28-F, 29-F, and 30-F. As stated by Solereder, the primary medullary rays are not closed by inter-fascicular wood. The length of medullary rays is somewhat greater in *B. vulgaris* than in *M. aquifolia*. The hybrid follows the *B. vulgaris* parent in this character. The vertical distance between rays is greater in *M. aquifolia* than in the *B. vulgaris*, and in this character the hybrid follows the *B. vulgaris* parent.

The pith of *M. aquifolia* is homogeneous and its walls are uniformly thickened throughout. The pith in *M. aquifolia* is never crushed, so far as I was able to observe. *B. vulgaris*, as observed by Solereder, has a heterogeneous pith with two areas, the outer thick-walled and remaining alive, while the inner thin-walled area soon dies (Pl. XL, Figs. 24 and 27, K and L). The hybrid has a hetero-

geneous pith similar to that found in the *B. vulgaris* parent, but the thick-walled area which remains alive is wider than in *B. vulgaris*. Compare Pl. XL, Figs. 23 and 26, of the hybrid with Figs. 24 and 27 K and L, of the *B. vulgaris* parent. The central dead area in the hybrid is smaller than in the *B. vulgaris* parent. This may be considered a bisexual inheritance in which the pith of the hybrid inherits characters partly from the *M. aquifolia* parent and partly from the *B. vulgaris* parent.

CONCLUSION.

The *Berberis neubertii* plant from which this study was made is undoubtedly a true hybrid of the *B. vulgaris* and *M. aquifolia* parents. It agrees with what we may expect from hybrids from previous studies. No new characters appear in the hybrid which do not occur in the parents.

B. neubertii is usually considered a bigeneric hybrid. In some characters it behaves as an interspecific hybrid, in others as an intervarietal hybrid, and in others as neither. It therefore belongs to the group which "affords no decision." (De Vries.)

Those characters common to one parent only, called unisexual by MacFarlane and De Vries, may behave as intermediates. The network of thickenings found on the lower epidermis of the *M. aquifolia* leaf, but not found on the leaf of *B. vulgaris,* are found in the hybrid much reduced.

The evergreen leaf of *M. aquifolia* and the deciduous leaf of *B. vulgaris* are inherited in the hybrid as semievergreen. The stiff xerophytic appearance of *M. aquifolia,* differing from the graceful, mesophytic appearance of *B. vulgaris,* is inherited as a semixerophytic appearance in the hybrid.

Papillose epidermal hairs found on *M. aquifolia* stems and lower epidermis of the leaf, but not on *B. vulgaris,* do not appear in the hybrid. Epidermal hairs in *Lonicera* in the hybrids studied behave as intermediates.

The amount of secondary bast in *Berberis neubertii* exceeds that found in the one parent only, the *B. vulgaris* parent.

Characters observed in the *B. vulgaris* parent only in which the hybrid closely approaches this parent are: spines, which are morphologically leaves, with the foliage leaves borne on short stems in their axils; jointed petiole; diagonal pits in the primary bast; the size, shape, and thickness of walls of secondary bast.

Characters observed in the *M. aquifolia* parent only in which the

hybrid closely approaches this parent are: second and third row of palisade cells of the leaf; the perforations and thickened walls of the cortex parenchyma; and certain deposits and longitudinal thickenings in the primary bast fibers.

Cases of bisexual heredity were not common in this hybrid, as they were not in those studied by MacFarlane. The heterogeneous pith of the *B. vulgaris* parent and the thickened-walled homeogeneous pith of *M. aquifolia* are both partly inherited by the hybrid. Some of the pith area in the hybrid shows thickening of cell walls and the central pith area is thin-walled as in *B. vulgaris*. Another example of apparent bisexual heredity is in the primary bast. Diagonal long pits are inherited from the *B. vulgaris* parent, and longitudinal thickenings and soluble deposits in the cell walls from the *M. aquifolia* parent. Both of these characters appear in the hybrid.

Bisexual characters are those common to both parents (MacFarlane and De Vries). Those characters in which the *M. aquifolia* parent dominates are: texture of the leaf, margin of the leaf, number of rows of palisade cells of the leaf, and number of pericycle parenchyma cells radially. Those characters in which the *B. vulgaris* parent dominates are: habit of indefinite growth; size of stipules; color, shape, and venation of the leaf; shape of guard cells; number of bundles in the midrib; general character of the cortex of the stem; features in the primary bast, cork, and parenchyma of the pericycle.

Many characters common to both parents behave as intermediates. The hybrid has an intermediate number of stomata of the leaf; length of epidermal hairs; length of cortex cells, wood fibers, and tracheids; amount of thickening of cell walls of parenchyma of the pericycle and septate phloem prosenchyma.

There are some characters common to both parents in which the hybrid exceeds both parents; as, size of the leaf epidermal cells, length of palisade cells of the leaf, number of schlerenchyma cells of the midrib transversely, thickening of the sides of the blade as compared with the midrib, number of primary bast fibers as shown by the number of rows counted radially, amount of wood parenchyma cells, and amount of sclerenchymatous medullary sheath abutting on the xylem in the stem.

A few characters common to both parents in which the hybrid has less than either parent are number of stomata of the stem and number of cells of the cortex radially.

While anatomical study increases greatly the number of characters observable, it also increases the complexity and difficulty of the study in its interpretation of the laws of heredity. It is generally recognized that microscopic details as well as macroscopic characters are heritable and it seems well established that they should be considered in any study of the transmission of characters in hybrids.

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PLATE XXXVII.

FIG. 1. Leaf of *Berberis vulgaris,* showing general type of venation. Drawn from leaf cleared with chloral hydrate. × 1.

FIG. 2. Leaf of *Berberis neubertii.* × 1.

FIG. 3. Leaflet of *Mahonia aquifolia.* × 1.

FIG. 4. Upper epidermis of leaf of *B. vulgaris,* showing nuclei and upper end of palisade cells. × 312.

FIG. 5. Upper epidermal cells of *B. neubertii,* showing nuclei and upper end of palisade cells. × 312.

FIG. 6. Upper epidermis of *M. aquifolia* leaf. × 312.

FIG. 7. Lower epidermis of leaf of *B. vulgaris,* showing stomata and nuclei. × 312.

FIG. 8. Lower epidermis of leaf of *B. neubertii,* showing stomata and nuclei, also network of thickenings. × 312.

FIG. 9. Lower epidermis of leaf of *M. aquifolia.* × 312.

FIG. 10. Two stomata of *B. vulgaris* in cross section, showing guard cells, epidermal cells, and air space. × 312.

FIG. 11. Two stomata of *B. neubertii.* × 312.

FIG. 12. Two stomata of leaf of *M. aquifolia.* × 312.

PLATE XXXVII.

PLATE XXXVIII.

FIG. 13. Cross section of leaf of *Mahonia aquifolia* near center of leaf. × 208.

FIG. 14. Cross section of leaf of *Berberis neubertii,* near center of leaf. × 208.

FIG. 15. Cross section of leaf of *B. vulgaris,* near center of leaf. × 208.

Index of parts of above drawings:

A, Upper epidermis.
B, Upper row of palisade cells.
C, Second row of palisade cells.
D, Third row of mesophyll showing gradual transition to spongy parenchyma.
E, Spongy parenchyma.
F, Lower epidermis.
G, Phloem of midrib bundle.
H, Xylem of midrib.
I, Cortex.
J, Air space.
K, Sclerenchyma cells forming bundle sheath.

FIG. 34. Phloem prosenchyma of *M. aquifolia* stem showing cell walls just beginning to thicken.

FIG. 35. Same as Figure 34, after cell walls have thickened and air spaces have formed between them.

FIG. 36. Cork A, phellogen B, parenchyma of pericycle C, all of *B. neubertii* showing perforations in the cell walls of the pericycle.

FIG. 37. Septate phloem prosenchyma of *B. neubertii* stem. Similar cells are found, also, in the phloem of *B. vulgaris.*

PLATE XXXVIII.

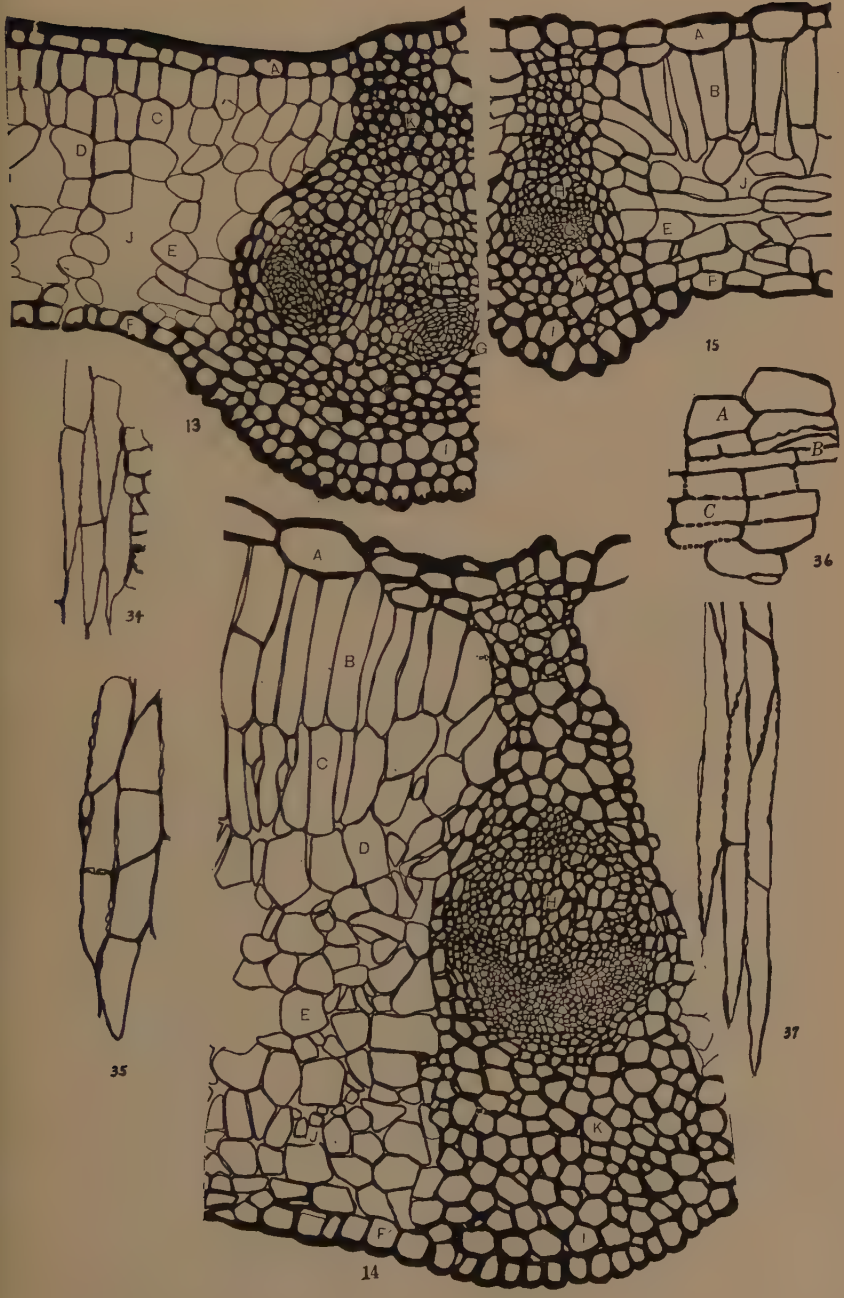


PLATE XXXIX.

FIG. 16. Cross section of second internode of rapidly growing stem of *Mahonia aquifolia.* × 115.

FIG. 17. Cross section of rapidly growing stem, second internode, of *B. neubertii.* × 115.

FIG. 18. Cross section of second internode of rapidly growing stem of *B. vulgaris.* × 115.

FIG. 19. Cross section of fifth internode of rapidly growing stem of *M. aquifolia.* × 150.

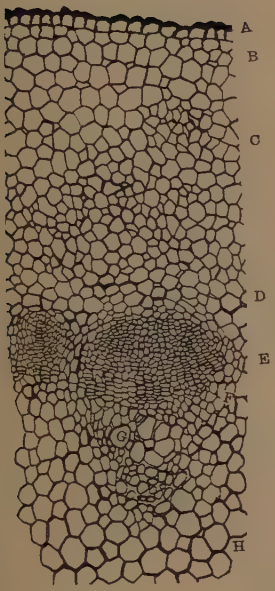
FIG. 20. Cross section of rapidly growing stem of *B. neubertii,* third internode. × 150.

FIG. 21. Cross section of rapidly growing stem of *B. vulgaris,* third internode. × 150.

Index for above figures:

A, Epidermis.
B, Parenchyma of the cortex.
C, Bast fibers or sclerenchyma of pericycle.
D, Parenchyma of the pericycle.
E, Primary and early secondary phloem.
F, Medullary ray.
G, Primary and early secondary xylem.
H, Pith.

PLATE XXXIX.



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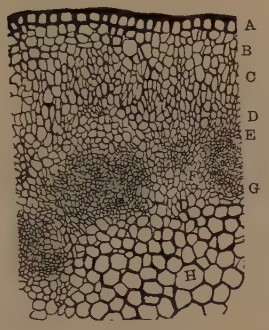

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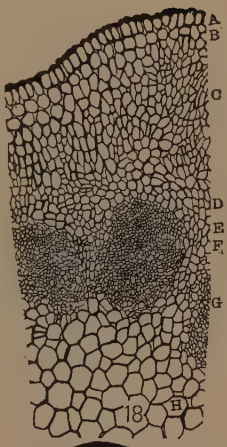

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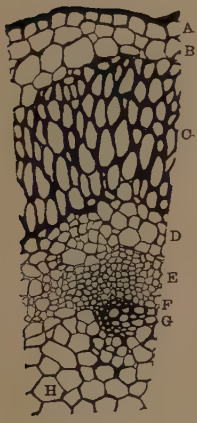

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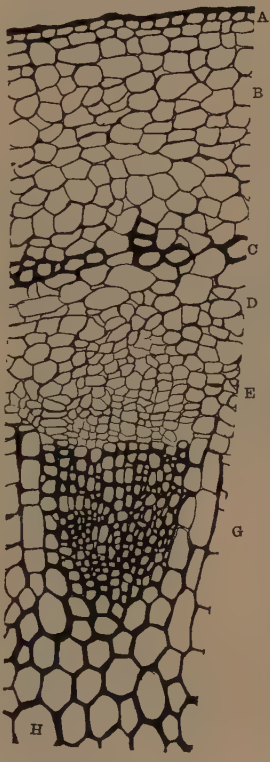

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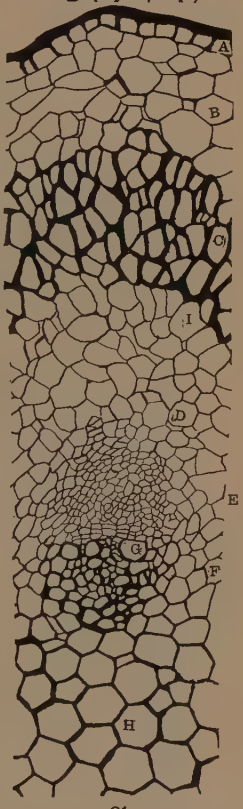

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PLATE XL.

FIG. 22. Cross section of one-year-old stem of *Mahonia aquifolia.* ×75.
FIG. 23. Cross section of same age of *Berberis neubertii* stem. ×75.
FIG. 24. Cross section of same age of stem of *B. vulgaris.* ×75.
FIG. 25. Cross section of two-year-old stem of *M. aquifolia.* ×75.
FIG. 26. Cross section of two-year-old stem of *B. neubertii.* ×75.
FIG. 27. Cross section of two-year-old stem of *B. vulgaris.* ×75.

Index for above figures:

A, Epidermis.
B, Parenchyma of the cortex.
C, Bast fibers or sclerenchyma of the pericycle.
D, Cork formed by the phellogen of the cork cambium.
E, Parenchyma of the pericycle.
F, Primary phloem.
G, Secondary phloem.
H, Medullary ray.
I, Secondary xylem.
J, Primary xylem.
K, Central pith. (In *B. vulgaris* and *B. neubertii* without thickened cell walls.)
L, Outer pith. (In *B. vulgaris* and *B. neubertii* with thickened cell walls.)
M, Phellogen or cork cambium.
N, Secondary bast just beginning to thicken its walls.
O, Secondary bast. (In Fig. 26, cross section. Fig. 27, longitudinal section.)

PLATE XL.

PLATE XLI.

FIG. 28. Tangential section from wood area of *Mahonia aquifolia* stem ×208.

FIG. 29. Tangential section from the wood area of *Berberis neubertii*. ×208.

FIG. 30. Tangential section from the wood area of *B. vulgaris*. ×208.

Index for above figures:

A, Primary xylem tracheal tube, showing thickening of cell wall.

B, Secondary xylem water tube, showing pits.

C, Water tubes of secondary xylem, showing pits and spiral thickenings.

D, Tracheids, showing bordered pits.

E, Wood fibers, showing simple pits.

F, Medullary rays in cross section, showing perforations.

G, Tracheal tube, showing spiral thickening in one part and simple pits in another. (Not common in this subject.)

FIG. 31. Individual primary bast fibers of *Mahonia aquifolia*, showing longitudinal thickenings and soluble deposits. ×208.

FIG. 32. Individual primary bast fibers of *B. neubertii*, showing longitudinal thickenings, soluble deposits, elongated pits, and circular pits. ×208.

FIG. 33. Individual primary bast fibers of *B. vulgaris*, showing elongated pits and circular pits. ×208.
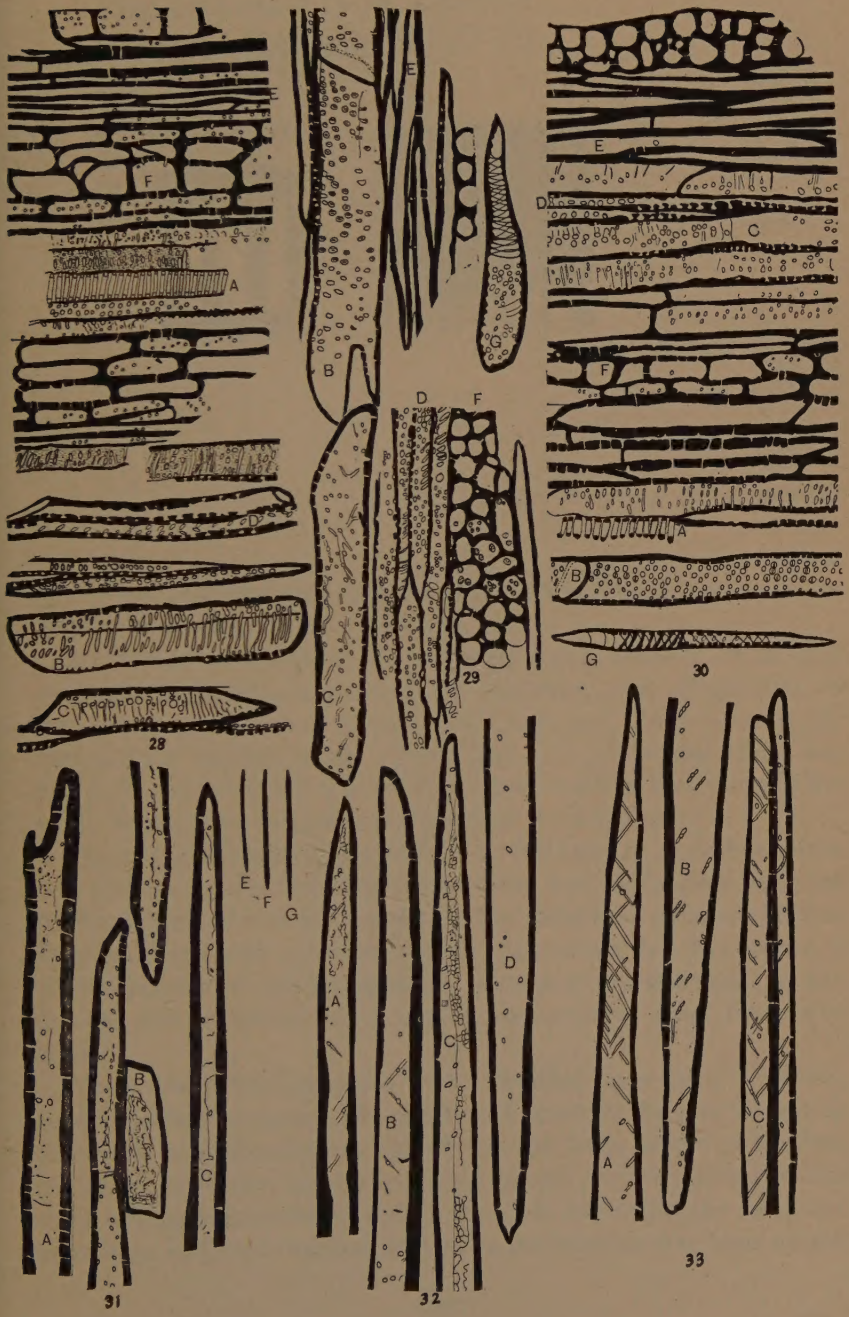
PLATE XLI.